90-06941/

PNFI Technical Reports

Petawawa National Forestry Institute, Chalk River, Ontario K0J 1J0, Canada

5

POLLINATION TECHNIQUES II. POLLEN EXTRACTION AND STORAGE

P.L. Copis
Forest Technologist
Tree Breeding and Genetics

INTRODUCTION

This is the second of three technical notes describing procedures used at the Petawawa National Forestry Institute (PNFI) in the production of control-pollinated seedlots. The present note addresses pollen handling, in particular, the low-cost techniques used at PNFI to extract and store pollen, and comments on pollen germination testing.

PROGRAM REQUIREMENTS

The pollen extraction techniques used at PNFI are intended for research operations involving 10-25 relatively small pollen lots and 50-100 crosses each year. The nature of the equipment used is simple, but the results are effective.

COLLECTING THE POLLEN FLOWER

At the time of collection, the microstrobili are picked and placed into a paper bag. With *Picea* and *Larix*, a section of twig or branch, usually bearing several microstrobili, is collected (Figure 1). Sometimes a large plastic garbage bag is required to accommodate the volume of branches collected. With *Pinus*, only the pollen flower cluster needs to be picked. The collection bags should be clearly labelled with an unique number to identify the source.

ROOM CONDITIONS

At the extraction facility, three separate rooms are used for the pollen-extraction process. In the first room (e.g. nursery rough lab), pollen flowers are spread onto an extraction tray or funnel and securely covered. Only one pollen lot is dealt with at a time. This is important to minimize contamination between pollen lots. Controlled room temperature and relatively humidity are not pressing concerns at this stage.

The extraction tray or funnel is then brought into a room (Figure 2) in which the controlled environment will promote the development of the microstrobili and the shedding of pollen. The room should be free of sunlight, have a relative humidity of 20-40%, and have a temperature of 25 ± 2°C. Good air circulation is required.

A separate room containing a fume hood, where pollen transfer takes place, is also used. Normal room conditions (temperature and light) are adequate in this room, but the relative humidity should preferably be kept within 20-40%.

POLLEN EXTRACTION

Seed-extraction tray

The traditional apparatus used for pollen extraction is a seed-extraction tray enveloped with brown wrapping paper (Figure 3). In the first room described earlier, the pollen flowers are placed onto these trays and the paper folds are sealed using masking tape. The tray is then placed on a table or shelf in the controlled-environment room (second room). The corners of the tray are elevated to allow air to circulate around the entire tray.

After the pollen has been released, the tray is gently carried to a fume hood located in a nearby room (third room). Here (and still sealed in paper), the tray is shaken and the pollen sifts through the fine mesh and onto the paper.

The paper containing pollen is then separated from the tray and the pollen is funnelled through a fine filter (panty hose works well) into a 50-100 mL vial. The vial is approximately one-third filled to allow the pollen to "breathe" and is then immediately put into a

desiccator. This ensures a moisture content that maintains the pollen in a usable condition (dry powdery state). Excessive moisture will cause the pollen to cake and potentially spoil, so proper room and storage conditions are essential.

Funnel extractor

A second system uses a 30 cm (12 in.) diameter metal gasoline funnel fitted with a removable fine mesh wire screen (used for seed extraction trays) on which the pollen flowers are placed (Figure 4). Between the screen and the funnel spout is a second wire screen having a finer mesh, which acts as a fine filter. Canvas or another pollen-impeding material is used to tightly cover the top of the funnel and a polyethylene or glass bottle is tightly attached over the funnel spout to collect the pollen. The funnel is then placed on a shelf in the controlled-environment room. Pollen handling is carried out under a fume hood and the pollen is put into desiccators as previously described.

A portable system (Figure 5) is made up of a 6 in. (15 cm) diameter metal funnel with a fine wire screen, an extended length of tight-fitting sausage casing and a 100 mL polyethylene collection bottle. The pollen flowers are collected and put into the assembled unit, ensuring that all potential leak points on the unit are sealed with masking tape. The unit is then placed in the controlled environment room. When the pollen flowers have opened, the bag is simply shaken and the pollen collects in the attached bottle. This system has been used effectively both locally and in remote field locations using a motel room as a laboratory. A desiccator is brought along to condition and store the pollen. Shed pollen is administered promptly to prevent problems with moisture pickup.

HYGIENE

Good hygiene is important to maintain the integrity of any pollen lot. From the time the pollen flowers are collected and up to pollen extraction, extreme care must be taken to negate free-flying pollen, which may contaminate pollen lots. This means dealing with one pollen lot at a time, ensuring that other pollen lots in the same room are covered, and very deliberate handling to avoid accidental emission of pollen. Also, after the pollen has been extracted and put into a desiccator, the equipment, spent pollen flowers, and fume hood have to be washed down with ethyl alcohol. This serves to decontaminate the fume hood prior to inserting the next pollen lot, and minimizes free-flying pollen in the room after the materials have been removed from the fume hood.

POLLEN STORAGE

Proper storage of pollen required for future use is essential in order to maintain viable pollen. For storage during the current pollination period, the vial is stopped with cotton and put into a desiccator. The desiccator is then placed in a cool dark room or a refrigerator. At the time the pollen is to be used, a required amount is funnelled (under the fume hood) into a pollen applicator container, placed into another desiccator, and taken to the field. If a refrigerator is used, the pollen is brought back to room temperature before the desiccator is opened.

For long-term storage (1 year or more), the desiccator is first placed inside a refrigerator (1 week is recommended) and then put into a chest freezer at approximately -18°C. At least two weeks prior to when the pollen is required the desiccator is taken from the freezer, put into a refrigerator for 24 hours, and then brought back to room temperature before being opened. At that time and before being used, a sample of pollen is taken for germination testing (see next section). Using this technique, pollen of *Pinus* and *Picea* has been used successfully in PNFI programs after three years or more in storage.

POLLEN GERMINATION TESTING

Owens and Blake (1985) indicate that germinability may not be an adequate measure of pollen "viability" and their literature review suggests that the rate of pollen tube growth should be employed as a measure of fertilizing potential. They also state, however, that germination tests will remain an useful standard technique. Notes are given below of techniques used in germination testing of the three genera *Pinus*, *Picea*, and *Larix*.

Pinus

Pollen grains (at room temperature) are lightly sprinkled into a vial of distilled water. The vials are repeatedly shaken to prevent the pollen from clumping. In 2-3 days, sample drops are placed onto slides, covered, and viewed under a dissecting microscope (X100 magnification). A minimum of two fields of 50 pollen grains are assessed to derive the per cent germination. Sucrose added to distilled water (or other germination medium) tends to promote pollen germination and development of the pollen tubes. A sucrose concentration of 10 per cent is the average recommendation for use

Figure 1. Collecting pollen

Figure 2. The controlled environment room

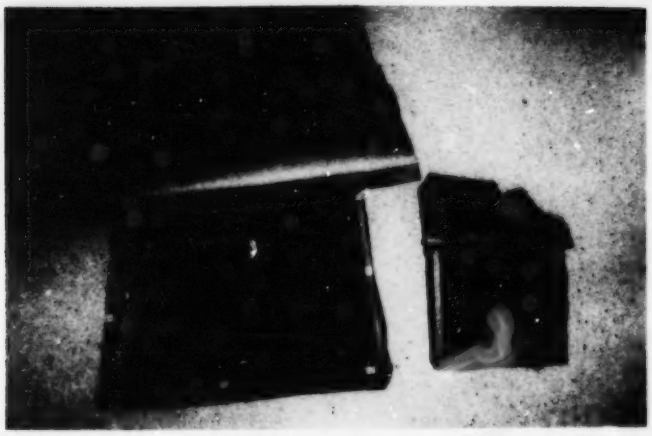

Figure 3. Seed extraction tray

Figure 4. The funnel extractor

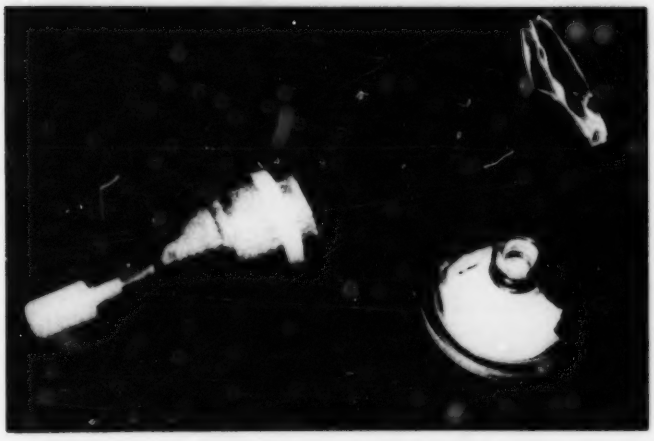

Figure 5. Portable funnel extractor system

(USDA Forest Service 1981). Adding sucrose in germination tests for pine pollen is not generally required since excellent results occur using distilled water alone.

Germination is considered to have taken place when elongation of the pollen tube exceeds the shortest dimension of the pollen grain. If the pollen tube has started to disintegrate, it is considered to have germinated.

Picea

Results obtained using the above technique with *Picea* species are generally poor and inconsistent. Instead, testing on a solid medium has been adopted (USDA Forest Service 1981, OMNR 1988). Repeatable results with high germination percentages for *Picea* as well as *Pinus* are achieved using the following technique:

A solution containing distilled water, 0.6% Phytagar (Gibco Laboratories), and 5% sucrose is put into a microwave at maximum power and allowed to boil for about 1 min. It is then cooled for 30 min., poured into small petri dishes such that the bottom is covered, and left to solidify. Pollen (conditioned to room temperature and humidity) is lightly dusted onto the surface of the medium. The petri dishes are covered and left in a warm room over saturated paper, away from sunlight. Germination counts are made within 24-48 hours using a dissecting microscope.

Rehydration of stored pollen prior to using the above method is described by the USDA Forest Service (1981). The purpose is to bring the moisture content to a level comparable with that of fresh pollen and to optimize germination results. For the purposes of PNFI programs suitable results are achieved using pollen reconditioned to room temperature and humidity.

Larix

In *Larix* species, pollen tubes do not develop in an artificial environment and germination counts are much more difficult (USDA Forest Service 1974). Germination testing of *Larix* pollen is not attempted at PNFI.

REFERENCES

OMNR (Ontario Ministry of Natural Resources). 1988. Pollen management workshop, Orono, Ontario.

Owens, J.N.; Blake, M.D. 1985. Forest tree seed production. Can. For. Serv. Inf. Rep. PI-X-53.

USDA (United States Department of Agriculture) Forest Service. 1974. Seeds of woody plants in the United States. Agric. Handb. 450.

USDA (United States Department of Agriculture) Forest Service. 1981. Pollen management handbook. Agric. Handb. 587.

1990

Canada

ISSN 1180-2618
ISBN 0-662-17945-5
Catalogue No. Fo29-26/5E

Forestry Canada Forêts Canada